NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF ETHYLBENZENE

(CAS NO. 100-41-4)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

January 1999

NTP TR 466

NIH Publication No. 99-3956

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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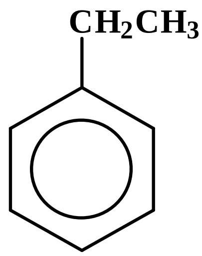
ABSTRACT



ETHYLBENZENE

CAS No. 100-41-4

Chemical Formula: C_8H_{10} Molecular Weight: 106.16

Synonyms: EB; ethylbenzol; phenylethane

Ethylbenzene is mainly used in the manufacture of styrene. Ethylbenzene is also a major component of mixed xylenes used as solvents in agricultural and home insecticide sprays, rubber and chemical manufacturing, and household degreasers, paints, adhesives, and rust preventives. Ethylbenzene is also used as an antiknock agent in aviation and motor fuels. Ethylbenzene was nominated for study by the National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) because of its potential for widespread human exposure and because of its structural similarity to benzene and toluene. Male and female F344/N rats and B6C3F_1 mice were exposed to ethylbenzene (greater than 99% pure) by inhalation for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, mouse lymphoma cells, cultured Chinese hamster ovary cells, and mouse peripheral blood erythrocytes. In previously reported 13-week toxicity studies in which F344/N rats and B6C3F_1 mice were exposed to ethylbenzene by whole body inhalation exposure, no histopathologic changes were observed (NTP, 1992).

2-Year Study in Rats

Groups of 50 male and 50 female F344/N rats were exposed to 0, 75, 250, or 750 ppm ethylbenzene by inhalation, 6 hours per day, 5 days per week, for 104 weeks.

Survival and Body Weights

Survival of male rats in the 750 ppm group was significantly less than that of the chamber controls. Mean body weights of 250 and 750 ppm males were generally less than those of the chamber controls beginning at week 20. Mean body weights of exposed groups of females were generally less than those of chamber controls during the second year of the study.

Pathology Findings

In male rats exposed to 750 ppm, the incidences of renal tubule adenoma and adenoma or carcinoma (combined) were significantly greater than the chamber control incidences. In addition, the incidence of renal tubule hyperplasia in 750 ppm males was significantly greater than that in the chamber controls.

The findings from an extended evaluation (step section) of the kidneys showed a significant increase in the incidences of renal tubule adenoma and hyperplasia in 750 ppm males and females; the incidence of renal tubule adenoma or carcinoma (combined) was significantly increased in 750 ppm males. The severities of nephropathy in 750 ppm male and all exposed female rats were significantly increased relative to the chamber controls.

The incidence of interstitial cell adenoma in the testis of 750 ppm males was significantly greater than that in the chamber control group and slightly exceeded the historical control range for inhalation studies.

2-Year Study in Mice

Groups of 50 male and 50 female B6C3F$_1$ mice were exposed to 0, 75, 250, or 750 ppm ethylbenzene by inhalation, 6 hours per day, 5 days per week, for 103 weeks.

Survival and Body Weights

Survival of exposed groups of male and female mice was similar to that of the chamber controls. Mean body weights of female mice exposed to 75 ppm were greater than those of the chamber controls from week 72 until the end of the study.

Pathology Findings

In 750 ppm males, the incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) were significantly greater than those in the chamber control group but were within the NTP historical control ranges. The incidence of alveolar epithelial metaplasia in 750 ppm males was significantly greater than that in the chamber controls.

In 750 ppm females, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly greater than those in the chamber control group but were within the historical control ranges. The incidence of eosinophilic foci in 750 ppm females was significantly increased compared to that in the chamber controls. There was a spectrum of nonneoplastic liver changes related to ethylbenzene exposure in male mice, including syncytial alteration of hepatocytes, hepatocellular hypertrophy, and hepatocyte necrosis.

The incidences of hyperplasia of the pituitary gland pars distalis in 250 and 750 ppm females and the incidences of thyroid gland follicular cell hyperplasia in 750 ppm males and females were significantly increased compared to those in the chamber control groups.

Genetic Toxicology

Ethylbenzene gave little indication of mutagenicity, *in vitro* or *in vivo*. No induction of mutations was noted in *Salmonella typhimurium* strain TA97, TA98, TA100, or TA1535 with or without S9 metabolic activation, and no increases in sister chromatid exchanges or chromosomal aberrations were observed in cultured Chinese hamster ovary cells treated with ethylbenzene, with or without S9. In the mouse lymphoma assay, a significant mutagenic response was noted in the absence of S9, but only at the highest nonlethal dose tested and with accompanying cytotoxicity; the test was not performed with S9. No increases in the frequency of micronucleated erythrocytes were observed *in vivo* in peripheral blood samples from male and female mice exposed to ethylbenzene for 13 weeks.

Conclusions

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity** of ethylbenzene in male F344/N rats based on increased incidences of renal tubule neoplasms. The incidences of testicular adenoma were also increased. There was *some evidence of carcinogenic activity* of ethylbenzene in female F344/N rats based on increased incidences of renal tubule adenomas. There was *some evidence of carcinogenic activity* of ethylbenzene in male B6C3F$_1$ mice based on increased incidences of alveolar/bronchiolar neoplasms. There was *some evidence of carcinogenic activity* of ethylbenzene in female B6C3F$_1$ mice based on increased incidences of hepatocellular neoplasms.

Exposure of male and female rats to ethylbenzene resulted in increased incidences of renal tubule hyperplasia and increased severities of nephropathy. Exposure of male mice to ethylbenzene resulted in increased incidences of alveolar epithelial metaplasia, syncytial alteration of hepatocytes, hepatocellular hypertrophy, hepatocyte necrosis, and thyroid gland follicular cell hyperplasia. In female mice, ethylbenzene exposure resulted in increased incidences of eosinophilic foci of the liver, pituitary gland pars distalis hyperplasia, and thyroid gland follicular cell hyperplasia.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Ethylbenzene

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Concentrations in air	Chamber control, 75, 250, or 750 ppm	Chamber control, 75, 250, or 750 ppm	Chamber control, 75, 250, or 750 ppm	Chamber control, 75, 250, or 750 ppm
Body weights	250 and 750 ppm groups less than chamber controls	Exposed groups less than chamber controls	Exposed groups similar to chamber controls	75 ppm group greater than chamber controls
Survival rates	15/50, 14/50, 13/50, 2/50	31/50, 31/50, 34/50, 35/49	28/50, 36/50, 32/50, 30/50	35/50, 38/50, 40/50, 37/50
Nonneoplastic effects	Kidney: renal tubule hyperplasia (standard evaluation - 2/50, 2/50, 4/50, 12/50; standard and extended evaluations combined - 11/50, 9/50, 11/50, 23/50); severity of nephropathy (2.3, 2.4, 2.3, 3.5)	Kidney: renal tubule hyperplasia (standard evaluation - 0/50, 1/50, 3/50, 3/49; standard and extended evaluations combined - 1/50, 2/50, 4/50, 10/49); severity of nephropathy (1.3, 1.6, 1.7, 2.3)	Lung: alveolar epithelial metaplasia (0/50, 1/50, 2/50, 6/50) Liver: syncytial alteration (0/50, 5/50, 8/50, 23/50); hypertrophy (1/50, 0/50, 0/50, 17/50); necrosis (1/50, 1/50, 3/50, 10/50) Thyroid gland: follicular cell hyperplasia (21/50, 21/50, 29/50, 32/50)	Liver: eosinophilic focus (5/50, 7/50, 6/50, 22/50) Pituitary gland (pars distalis): hyperplasia (10/48, 12/49, 23/47, 22/49) Thyroid gland: follicular cell hyperplasia (18/50, 23/50, 25/50, 35/50)
Neoplastic effects	Kidney: renal tubule adenoma (standard evaluation - 0/50, 3/50, 2/50, 4/50; standard and extended evaluations combined - 3/50, 5/50, 7/50, 20/50); renal tubule adenoma or carcinoma (standard evaluation - 0/50, 3/50, 3/50, 7/50; standard and extended evaluations combined - 3/50, 5/50, 8/50, 21/50) Testes: adenoma (36/50, 33/50, 40/50, 44/50)	Kidney: renal tubule adenoma (standard evaluation - 0/50, 0/50, 0/50, 1/49; standard and extended evaluations combined - 0/50, 0/50, 1/50, 8/49)	Lung: alveolar/bronchiolar adenoma (5/50, 9/50, 10/50, 16/50); alveolar/bronchiolar adenoma or carcinoma (7/50, 10/50, 15/50, 19/50)	Liver: hepatocellular adenoma (6/50, 9/50, 12/50, 16/50); hepatocellular adenoma or carcinoma (13/50, 12/50, 15/50, 25/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Ethylbenzene (continued)

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Level of evidence of carcinogenic activity	Clear evidence	Some evidence	Some evidence	Some evidence

Genetic toxicology	
Salmonella typhimurium gene mutations:	Negative in strains TA97, TA98, TA100, and TA1535 with and without S9
Mouse lymphoma gene mutations:	Positive without S9
Sister chromatid exchanges	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Chromosomal aberrations	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Micronucleated erythrocytes	
Mouse peripheral blood *in vivo*:	Negative

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on ethylbenzene on 11 and 12 December 1996 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 11 and 12 December 1996, the draft Technical Report on the toxicology and carcinogenesis studies of ethylbenzene received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. P.C. Chan, NIEHS, introduced the toxicology and carcinogenesis studies of ethylbenzene by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions were *clear evidence of carcinogenic activity* in male F344/N rats and *some evidence of carcinogenic activity* in female F344/N rats and male and female B6C3F$_1$ mice.

Dr. Reddy, a principal reviewer, agreed with the proposed conclusions. He said that for the purpose of contrasting findings with those of Maltoni *et al.* (1985), the Technical Report should cite information on types, sites, and incidences of neoplasms from that study. Dr. Chan said that in that study, the total number of neoplasms was provided but not differentiated by target organ. Dr. Reddy noted that the methods, such as immunochemistry, used to rule out $\alpha 2\mu$-globulin nephropathy in male rats should be described in the Technical Report. Dr. J. Mahler, NIEHS, responded that the hematoxylin-eosin stain, a good screen for hyaline droplet accumulation, was used.

Dr. Goldsworthy, the second principal reviewer, agreed with the proposed conclusions for rats and female mice. He agreed that the inhalation route was appropriate, but he noted that ethylbenzene has been detected in surface and ground water. Dr. Goldsworthy thought that the additional information obtained from renal step sections was helpful but asked for justification of the decision to step section kidneys but not other organs, such as thyroid and pituitary glands. Dr. J.R. Hailey, NIEHS, said that the major reason to step section organs is to help interpret equivocal or uncertain effects, and that endocrine organs such as thyroid and pituitary glands are too small to step section. Dr. Goldsworthy suggested that *clear evidence of carcinogenic activity* may have been a better call in male mice, based on a positive exposure-response trend and the presence of metaplasia in the target tissue. Dr. Mahler said that metaplasia is an unusual lesion and is generally not recognized as a precursor to neoplasia.

Dr. Ryan, the third principal reviewer, agreed with the proposed conclusions for rats. She said that one of the reasons for studying the chemical was its structural similarity to benzene and toluene, and she questioned why the Technical Report did not include more discussion comparing the toxic effects of the three chemicals (see Table 12, page 49). She expressed concern that the 750 ppm exposure in female rats and in male and female mice may have been too low because there were no survival or body weight effects in these groups. Dr. J.R. Bucher, NIEHS, commented that prechronic studies were performed with ethylbenzene and that an NTP study report was published in 1992. Because there were essentially no histopathologic findings in the 13-week studies, the exposure selection for the 2-year study was based on a body weight deficit in male rats. Dr. Ryan said that it could be argued that there was *clear evidence of carcinogenic activity* in male mice based on an exposure-related increase in combined benign and malignant lung neoplasms and in female mice based on an exposure-related increase of combined benign and malignant hepatic neoplasms. Dr. J.K. Haseman, NIEHS, said that there were three reasons for the level of evidence chosen: first, the neoplasm rates fell within the historical control range; second, the neoplasms were primarily benign; and third, the lung neoplasms were seen only in males and the liver neoplasms only in females.

Dr. LeBoeuf commented that survival in 750 ppm male rats was only 4% but the level of evidence of carcinogenic activity in male rats was based on increased incidences of renal tubule neoplasms in the 750 ppm group. He said that he was uncomfortable basing the level of evidence of carcinogenic activity on findings accompanied by such poor survival. Dr. Bucher responded that the fact that increased renal neoplasms were seen in both males and females and were accompanied by severe nephropathy, which is rarely if ever seen in females, suggests an intrinsic carcinogenic activity of ethylbenzene.

Dr. Ryan moved that the Technical Report on ethylbenzene be accepted with the revisions discussed and the conclusions as written for male rats, *clear evidence of carcinogenic activity*, and for female rats and male and female mice, *some evidence of carcinogenic activity*. Dr. Reddy seconded the motion, which was accepted unanimously with nine votes.

Later in the meeting, Dr. LeBoeuf made a motion to reopen the discussion on the neoplasm response in male rats. Dr. Taylor thought that the maker and seconder of the original motion should have to agree. Drs. Ryan and Reddy agreed to reopen the discussion. Dr. Goldsworthy seconded the motion to reopen the discussion, which was accepted by six yes votes to two no votes (Drs. Brown and Reddy). Dr. Ward was not present.

Dr. LeBoeuf stated that his primary concerns were the mortality in 750 ppm male rats and the interpretation of the data at that dose. He said that one of the original National Cancer Institute guidelines for the 2-year bioassays is that particular treatments should not affect survival, unless reduced survival is a result of neoplasia, and should not cause more than a 10% decrease in body weight gain. He said that in the ethylbenzene Technical Report, it was clear that the majority of the neoplasms in male rats were considered to be incidental to the cause of death. For this reason, he recommended changing the conclusion in male rats to *some evidence of carcinogenic activity*. Dr. Haseman pointed out that at week 84, the survival in 750 ppm male rats was still 70%. Dr. Goldsworthy stated that one issue to consider is when the first neoplasms arose. Dr. Bucher commented that nephropathy was likely the primary contributor to mortality. Dr. Haseman suggested that the conclusion for male rats, as with the report on oxazepam, could indicate that there was *clear evidence of carcinogenic activity* only at concentrations resulting in enhanced nephropathy. Dr. Bucher noted that in many past studies, the conclusions for carcinogenic activity were confirmed even when the maximum tolerated doses were exceeded. He noted that in most studies in which renal tubule neoplasms are associated with nephropathy in male rats, carcinomas are generally not seen; he further noted that in female rats, the incidences of nephropathy are generally less than in male rats and that 21 neoplasms is exceptionally high. Dr. Goldsworthy reminded the reviewers of the stipulation "Under the conditions of these studies..." Dr. Ryan pointed out that in the standard evaluation, the renal tubule neoplasm incidences in male rats exceeded the historical control range even in the 75 ppm group.

Dr. LeBoeuf moved that the conclusion for male rats be changed to *some evidence of carcinogenic activity*. Dr. Ryan seconded the motion, which was defeated by six no votes to two yes votes (Drs. LeBoeuf and Russo). Dr. Ward was not present.